

# Prognostic Value of Epicardial Coronary Artery Constriction to the Cold Pressor Test in Type 2 Diabetic Patients With Angiographically Normal Coronary Arteries and No Other Major Coronary Risk Factors

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**OBJECTIVE**— Endothelium-dependent coronary dilation is impaired in diabetic patients and has been found to independently predict cardiovascular events (CVEs) in patients with multiple coronary risk factors. The aim of this study was to evaluate the outcome of type 2 diabetic patients on the basis of epicardial coronary dysfunction.

**RESEARCH DESIGN AND METHODS**— We examined 56 control subjects (aged  $51.7 \pm 6.4$  years) using coronary artery response to the cold pressor test (quantitative coronary angiography) and compared them with 72 type 2 diabetic patients (aged  $50.3 \pm 8.5$  years) without other major coronary risk factors.

**RESULTS**— Average diameter change was  $17.2 \pm 10.4\%$  in the control subjects, dilation occurred in 91.1% of subjects, no change occurred in 8.9%, and there was no constriction. Average diameter change was  $-14.4 \pm 12.1\%$  in diabetic patients ( $P < 0.001$  vs. control subjects), constriction occurred in 73.6%, no change occurred in 26.4%, and there was no dilation. CVEs were recorded with a mean follow-up of  $45 \pm 19$  months. There was 1 CVE in the control group and 26 CVEs in 18 of 72 diabetic patients ( $P < 0.001$  vs. control subjects), with 23 events in 16 of 53 diabetic patients with coronary artery constriction ( $P < 0.001$  vs. control subjects), and 3 events in 2 of 19 diabetic patients with no diameter change (NS vs. control subjects).

**CONCLUSIONS**— In type 2 diabetic patients without other major coronary risk factors, constriction of angiographically normal coronary arteries to the cold pressor test is predictive of long-term CVEs.

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**Abbreviations:** CPT, cold pressor test; CVE, cardiovascular event.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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In diabetic patients, coronary atherosclerosis develops earlier than in other subjects and accounts for excessive morbidity and mortality (1). Because it is well established that the endothelium plays a key role in the regulation of vascular tone and the development of atherosclerosis (2), it has been suggested that endothelial dysfunction could be a predictor of cardiovascular risk (3). It has been shown that endothelium-dependent epicardial coronary artery vasodilation in response to acetylcholine (4) or physiological stimuli (5) is impaired in diabetic patients, suggesting that endothelial dysfunction occurs before the development of overt atherosclerosis. Recently, coronary endothelial dysfunction, evidenced by intracoronary acetylcholine, has been demonstrated to be an independent predictor of cardiovascular events (CVEs) in patients with coronary artery disease (6–8) and patients with coronary risk factors (8). The cold pressor test (CPT), which activates the sympathetic nervous system, induces dilation of coronary arteries in control subjects and constriction of atherosclerotic coronary arteries (6,9,10). In diabetic patients, CPT has been shown to induce coronary artery constriction (5), which may reflect endothelial dysfunction.

The purpose of the present study was to evaluate the ability of the epicardial coronary response to the CPT to predict CVEs in type 2 diabetic patients with angiographically normal coronary arteries and no other major risk factors.

## RESEARCH DESIGN AND METHODS

The study population was comprised of 56 control subjects and 72 type 2 diabetic patients undergoing diagnostic coronary arteriography between October 1994 and May 2000 because of

**Table 1—Clinical characteristics of the study group**

	Control subjects	Diabetic patients
n	56	72
Age (years)	51.7 ± 6.4	50.3 ± 8.5
Sex (male/female)	45/11	38/34*
Mean duration of diabetes (years)	—	10.7 ± 8.4
HbA <sub>1c</sub> (%)	—	6.9 ± 1.4
BMI (kg/m <sup>2</sup> )	26.1 ± 5.3	26.7 ± 4.6
Heart rate (bpm)	72 ± 10	77 ± 13
Systolic blood pressure (mmHg)	124 ± 10	136 ± 11†
Diastolic blood pressure (mmHg)	79 ± 7	83 ± 8
Total cholesterol (mmol/l)	5.10 ± 0.52	5.35 ± 0.57
Triglycerides (mmol/l)	1.35 ± 0.20	1.44 ± 0.38
HDL cholesterol (mmol/l)	1.73 ± 0.45	1.54 ± 0.38
LDL cholesterol (mmol/l)	3.12 ± 0.42	3.34 ± 0.45
End-diastolic diameter (mm)	51.2 ± 4.3	49.8 ± 5.7
Fractional shortening (%)	39.3 ± 5.5	37.5 ± 5.9
Left ventricular mass index (g/m <sup>2</sup> )	87 ± 12	90 ± 14
Creatinine clearance (ml/min)	111 ± 14	104 ± 21

Data are means ± SD. End-diastolic diameter, fractional shortening, and left ventricular mass index denote left ventricular echocardiographic measurements. \* $P < 0.001$ , † $P < 0.05$  vs. control subjects.

symptoms suggestive of angina, and/or an equivocal treadmill exercise test or single-photon emission computed tomography stress thallium scintigraphy. We excluded patients with other major coronary risk factors, including arterial hypertension, total cholesterol serum level (without treatment or with lipid-lowering therapy)  $>5.70$  mmol/l (220 mg/dl) or LDL cholesterol  $>3.70$  mmol/l (143 mg/dl), smoking, obesity (BMI  $\geq 30$  kg/m<sup>2</sup>), family history of premature coronary artery disease (defined as a first-degree relative aged  $<60$  years with clinical evidence of coronary atherosclerosis), aged  $>65$  years, and postmenopausal women without substitutive hormonal therapy. Patients with recent myocardial infarction, unstable angina, valvular heart disease, and atrial fibrillation were also excluded. None of the diabetic patients were insulin treated (13 sulfonylurea, 14 biguanide, 21  $\alpha$ -glucosidase inhibitor, 14 sulfonylurea + biguanide, and 10 biguanide +  $\alpha$ -glucosidase inhibitor), and all included patients were free of cardiovascular medication with the exception of short-acting nitrates, which were withdrawn the day before the investigation. All patients had normal left ventricular dimensions, mass, and systolic function, assessed by two-dimensional and M-mode echocardiography (11,12). Inclusion in the study was made with the consensus of two experienced investigators on immediate review

of the angiograms whenever coronary arteries were angiographically normal without luminal irregularities. The study protocol was approved by an institutional review committee.

Patients were studied in the fasting state and no premedication was administered. Hemodynamic measurements and a left coronary angiogram (baseline) were recorded 15 min after diagnostic coronary arteriography. Five minutes later, the CPT was performed. The patient's hands were immersed in ice water for 120 s, and the data were recorded immediately before removal of the hands from the ice water. At the end of the protocol, endothelium-independent coronary artery dilation was studied using intracoronary injection of a bolus of 2 mg isosorbide dinitrate.

#### Quantitative coronary arteriography

Measurement of the left anterior descending coronary artery diameter was performed by a previously validated technique (13). The accuracy of the technique was  $3.6 \pm 0.5\%$  (means ± SD) and the precision  $2.4 \pm 0.9\%$ . The maximum error between the actual and the calculated diameter was equal to  $\pm 5.7\%$  ( $R^2 = 0.994$ ). A segment of the guiding catheter filled with saline was placed close to the center of the image and used as a scaling device for calibration before beginning the procedure. Each angiogram was ana-

lyzed at random without knowledge of the sequence (baseline, CPT, and isosorbide dinitrate).

#### Long-term follow-up

Cardiovascular events were defined as sudden cardiac death, myocardial infarction, and stable and unstable angina documented by coronary arteriography, coronary angioplasty or surgical revascularization, stroke, and transient ischemic attack. For patients who were followed in the institution, hospital medical records were reviewed concerning the occurrence of cardiac events. Patients who were not followed in the institution were contacted by telephone. The study group was formed after exclusion of six patients (four control subjects and two diabetic patients) because follow-up could not be obtained.

#### Statistical analysis

All data are means ± SD. Differences between the groups for clinical and biological characteristics and basal hemodynamic parameters were compared with the nonparametric Mann-Whitney test. Differences between baseline and CPT hemodynamic parameters were compared with paired Student's *t* test. Comparisons between coronary artery dimensions at baseline, during CPT, and after intracoronary isosorbide dinitrate were made by two-way ANOVA with repeated measures for the experimental condition factor, followed by the Fisher protected least-significance difference test. The differences between the groups for CVE were analyzed using Pearson's  $\chi^2$  test. Cumulative event rates were evaluated by Kaplan-Meier survival curves. Probability values for survival curve comparisons were calculated with the log-rank statistic. The potential effect of therapy and patient variables were analyzed using Cox regression techniques. Statistical significance was assumed when  $P < 0.05$ .

**RESULTS**— The mean follow-up was  $45 \pm 19$  months (range 27–68). Patient characteristics are summarized in Table 1, which shows that there were no differences between the two groups for age, lipid profile, BMI, or echocardiographic parameters. However, the LDL-to-HDL ratio was higher in diabetic patients than in control subjects ( $2.17 \pm 0.33$  vs.  $1.80 \pm 0.24$ , respectively,  $P < 0.05$ ). Creatinine clearance was estimated using the

**Table 2—Hemodynamic data during the procedure**

	Control subjects		Diabetic patients	
	Baseline	CPT	Baseline	CPT
Heart rate (bpm)	70 ± 13	77 ± 15*	76 ± 15	78 ± 19
Aortic systolic pressure (mmHg)	126 ± 13	158 ± 21*	135 ± 19	168 ± 16*
Mean aortic pressure (mmHg)	97 ± 8	124 ± 13*	102 ± 12	134 ± 15*
Rate-pressure product (bpm · mmHg/10 <sup>2</sup> )	90.2 ± 17.9	120.1 ± 18.5*	101.4 ± 20.1	132.6 ± 19.3*

Data are means ± SD. \*P < 0.001 vs. baseline.

Cockcroft-Gault formula (14) and was similar in the two groups. The proportion of women was significantly higher in the diabetic group, and systolic pressure, although within the normal range according to the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure recommendations at the date of inclusion of the patients, was slightly higher in diabetic patients than in control subjects. Twelve control subjects and 43 diabetic patients were receiving a lipid-lowering therapy (statin, gemfibrosil, or fibrate).

**CPT and coronary artery changes**

The hemodynamic changes during CPT were comparable in both groups. Aortic pressures increased significantly in both groups. Although heart rate did not increase in diabetic patients and was slightly increased in control subjects, rate-pressure product increase was comparable in both groups (Table 2).

The coronary artery responses to the CPT were defined as dilation (change ≥6%), no change (change <±6%), and constriction (change ≥−6%). These values were chosen according to the highest error reported in the validation technique, i.e., 5.7%, a change in vessel diameter being defined as a minimum 6% variation. Values of coronary artery diameter throughout the procedure are provided in Table 3, which shows that baseline diameters were similar in diabetic patients and control subjects.

In control subjects, dilation occurred in 51 of 56 subjects, and no change occurred in 5 of 56 subjects. Constriction was never observed in these subjects (Fig. 1). Constriction was, however, observed in most of the diabetic patients (53 of 72); in 19 of 72 diabetic patients no diameter change was observed, and none of the pa-

tients showed significant dilation. The average diameter change (Fig. 1) was significantly higher (17.2 ± 10.4%) in control subjects than in diabetic patients (−14.4 ± 12.1%). According to the response, diabetic patients were divided into two groups, those with no change constituted group 1 (n = 19, average diameter change 2.7 ± 4.3%), and those with constriction constituted group 2 (n = 53, average diameter change −19.8 ± 5.6%).

An intracoronary injection of isosorbide dinitrate, an endothelium-independent coronary artery dilator, showed comparable results in all the groups (Fig. 1): 26.4 ± 11.8% in control subjects, 24.9 ± 14.3% in diabetic patients, 25.2 ± 11.4% in group 1, and 23.5 ± 12.7% in group 2.

**Cardiovascular events during follow-up**

Cardiovascular events are summarized in Table 4. In control subjects, only one transient ischemic attack was observed. Comparing the number of subjects with events, results show that CVEs were significantly higher in diabetic patients (26 for 18 patients) (Fig. 2). Frequency of CVE was not different in group 1 (three in two patients) and control subjects. Con-

versely, the frequency of CVEs was significantly higher in group 2 (23 in 16 patients) than in control subjects (Fig. 2). In addition, the most severe and acute CVEs (stroke, sudden cardiac death, unstable angina, and myocardial infarction) were observed only in group 2. Analysis of the cumulative proportion of CVE-free survivals shows that in diabetic patients with constriction, the incidence of events was significantly higher than in control subjects (Fig. 2), as opposed to diabetic patients without constriction. Moreover, the first CVE occurred 19 months after the inclusion in group 2 and 44 months after inclusion in group 1. Finally, there was a trend toward more constriction of coronary arteries in the 18 patients with (−22.7 ± 14.9%) than in the 54 patients without (−13.5 ± 12.7%) CVEs.

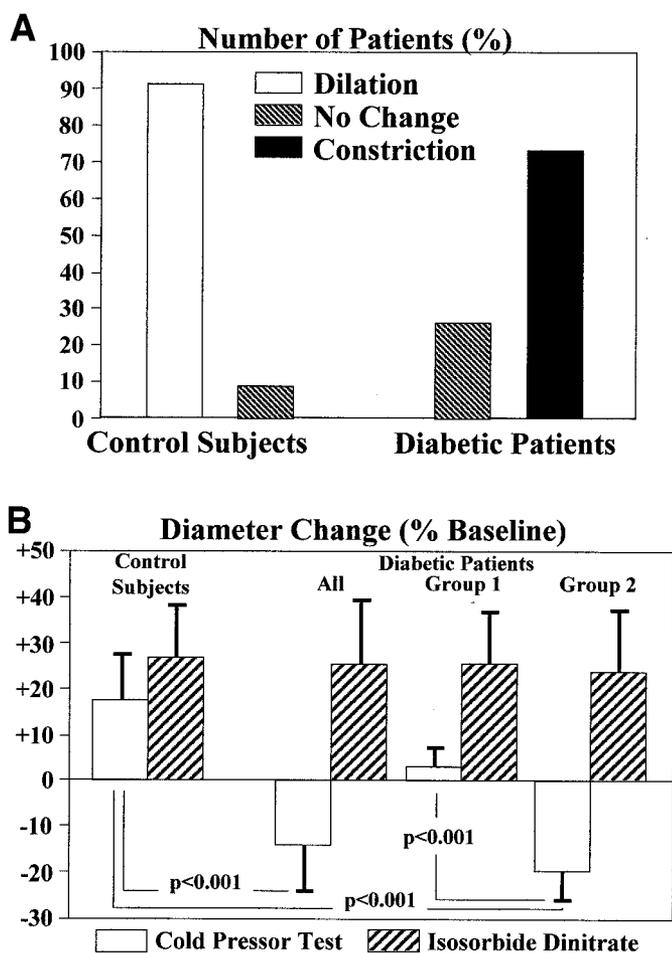
On the other hand, none of the control subjects had cardiac events (Table 3 and Fig. 3), whereas the patients in group 2 had most of them (16 in 11 patients) compared with group 1 (2 in 1 patient) (Fig. 3). The analysis of the cumulative proportion of cardiac event-free survivals shows that in group 2, the number of cardiac events was significantly higher than in control subjects, and the first cardiac event occurred 27 months after inclusion. Conversely, in group 1, the number of cardiac events was comparable to control subjects, and the first one occurred after a 57-month delay (Fig. 3).

The Cox regression analysis shows that the CVE rate differed between group 2 and control subjects while adjusting for differences in baseline clinical characteristics (age, sex, BMI, arterial pressure, and lipid profile). On the other hand, there was no difference between the clinical characteristics of the two groups of diabetic patients (age, sex, BMI, arterial pressure, lipid profile, duration of diabetes, and HbA<sub>1c</sub>). Last, the frequency of use of the different classes of antidiabetic (sulfonylureas, biguanides,

**Table 3—Coronary artery diameters (mm) during the procedure**

	Baseline	CPT	Isosorbide dinitrate
Control subjects (n = 56)	3.62 ± 0.42	4.24 ± 0.48*	4.58 ± 0.45*
Diabetic patients (n = 72)	3.51 ± 0.45	3.00 ± 0.37*	4.38 ± 0.68*
Diabetic patients with no change (group 1, n = 19)	3.52 ± 0.41	3.64 ± 0.42	4.41 ± 0.57*
Diabetic patients with constriction (group 2, n = 53)	3.47 ± 0.46	2.80 ± 0.36*	4.29 ± 0.73*

Data are means ± SD. \*P < 0.001 vs. baseline.



**Figure 1**—A: Types of responses of left anterior descending coronary artery to the CPT in the study population. B: Diameter changes in each group of patients in response to the CPT and intracoronary isosorbide dinitrate.

and  $\alpha$ -glucosidase inhibitors) and lipid-lowering agents was not different in these two groups, and there was no difference in the duration of diabetes.

**CONCLUSIONS**— The main findings of the present study conducted on type 2 diabetic patients with angiographically normal coronary arteries is that coronary artery constriction induced by CPT is predictive of CVEs and cardiac events. Because CPT-induced coronary constriction has been demonstrated to be due to endothelial dysfunction in patients with risk factors (5,10,15,16), patients with other major confounding factors have been excluded from this study, which clearly demonstrates the role played by type 2 diabetes per se. In addition, this study shows that response to nonpharmacological testing achieved during coronary angiography, which does not require intracoronary Doppler measurements, can be predictive of CVEs in diabetic patients.

#### CPT response as a witness of coronary endothelial dysfunction

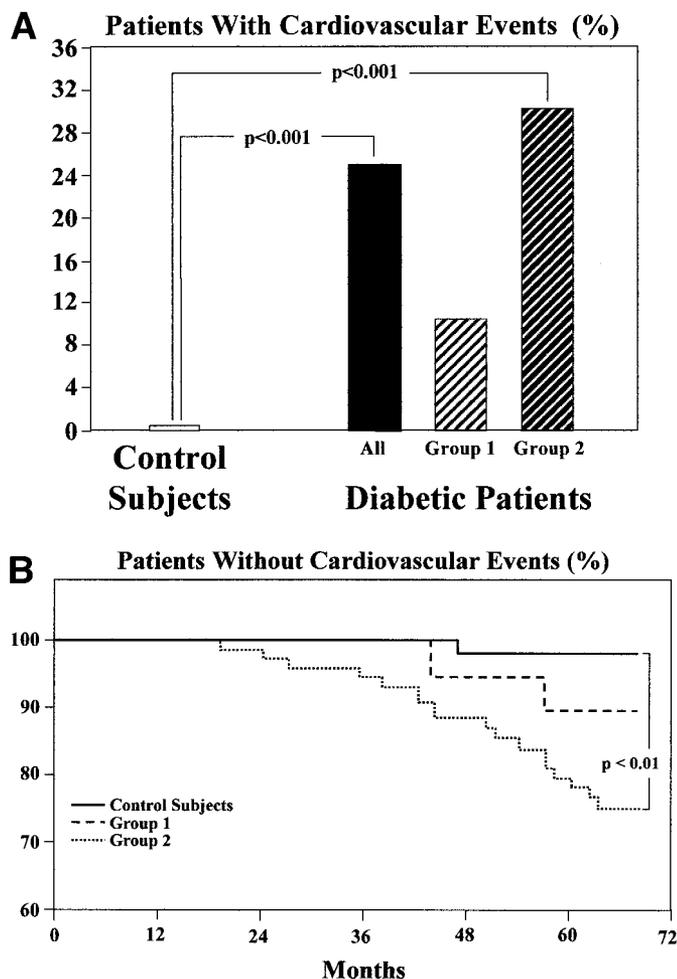
In normal subjects, the CPT induces sympathetic stimulation (17), thus increasing heart rate, arterial blood pressure (9,10), and myocardial oxygen demand. Enhanced myocardial metabolic demand re-

sults in an increase in coronary blood flow and dilation of epicardial coronary arteries (18). Dilation of coronary arteries is endothelium dependent and mainly due to the release of nitric oxide secondary to the stimulation of  $\alpha_2$ -adrenoceptors located on endothelial cells (19) and to flow-dependent dilation (20,21). Thus, the endothelium facilitates vasodilation of both resistance and large coronary vessels (22,23). Conversely, CPT constricts atherosclerotic coronary arteries (9,24) as well as both coronary arteries and microcirculation in patients with risk factors but normal arteries (5,10,25,26).

In this study, CPT induced constriction of epicardial coronary arteries in most diabetic patients, whereas the magnitude of dilation of the arteries of control subjects was comparable to that reported in other studies (9,24). The resistance of the coronary arteries to relaxing in diabetic patients could be caused by endothelial dysfunction so that flow-dependent dilation is blunted and reveals adrenergic vasoconstriction. It has been suggested that oxidative stress, which is increased in diabetes (27), can depress endothelium-mediated dilation by inactivation of nitric oxide (28). Indeed, an antioxidant agent could restore endothelium-dependent dilation in diabetes (5,29,30). On the other hand, it has been shown that there was a failure of coronary blood flow to adequately increase in diabetic patients because of impaired metabolic microvascular dilation (26,31), which could mask the ability of epicardial arteries to dilate in the absence of flow velocity increase. In the present study, coronary blood flow was not measured, and we cannot conclude whether the impairment of coronary artery dilation was

**Table 4**—Cardiovascular events during follow-up

	Control subjects	Diabetic patients	
		Group 1	Group 2
n	56	19	53
Sudden cardiac death	0	0	1
Myocardial infarction	0	0	3
Unstable angina	0	0	3
Stable angina	0	1	3
Coronary angioplasty	0	1	4
Surgical revascularization	0	0	2
Stroke	0	0	4
Transient ischemic attack	1	1	3
Total events	1	3	23



**Figure 2**—A: Proportion of patients with CVEs in each group of the study population. B: Proportion of patients free of CVEs in the different groups (Kaplan-Meier analysis, log-rank P value).

primarily due to large vessel dysfunction or the consequence of the absence of coronary blood flow increase due to microvascular dysfunction. However, it must be pointed out that even when flow-dependent dilation is abolished, coronary arteries do not constrict (5,32). Thus, whatever the mechanism, constriction of epicardial arteries in diabetic patients reflects an endothelial dysfunction. Then again, lipid-lowering therapy is known to affect endothelial function and restore normal dilation in hypercholesterolemic patients. In our study, we have not found differences between patients taking lipid-lowering therapy and the other subjects at baseline in the control group, where no patient evidenced coronary artery constriction to the CPT, and in the two diabetic subgroups, where the proportion of patients taking lipid-lowering therapy was comparable in both groups (12 of 19 in group 1 and 31 of 53 in group 2). Fi-

nally, because the rate-pressure product was similarly increased in control subjects and diabetic patients (Table 2), a lower metabolic stimulus cannot explain the coronary constriction in the latter.

Because coronary artery diameter changes in response to an exogenous donor of nitric oxide, isosorbide dinitrate, were comparable in control subjects and the two groups of diabetic patients, an impaired responsiveness of underlying vascular smooth muscle cells or structural changes of the vascular wall can also be ruled out.

#### Prediction of CVEs in type 2 diabetic patients

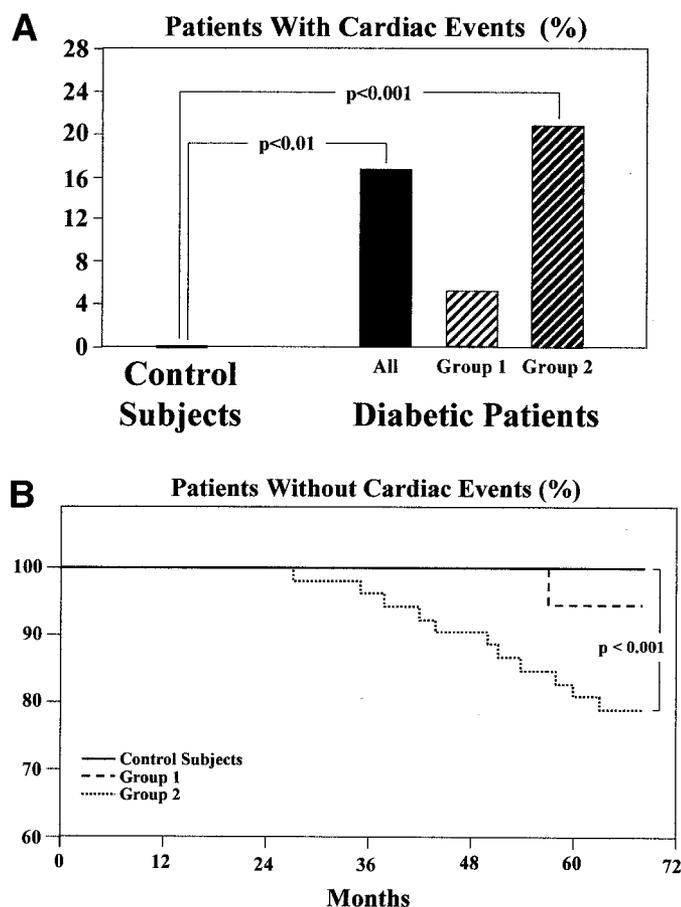
Recent studies have provided conflicting results about cardiovascular risk in type 2 diabetes. Haffner et al. (33) have shown that diabetic patients without myocardial infarction have as high a risk of coronary death as nondiabetic patients with prior myocardial

infarction. However, Evans et al. (34) concluded that type 2 diabetic patients were at lower risk for CVEs than patients with coronary artery disease. Thus, prediction of CVE with special attention to major cardiac events is an important challenge in type 2 diabetic patients, who are frequently asymptomatic. Although the exercise stress test and thallium-201 single-photon emission computed tomography have been demonstrated to be predictive in high-risk patients (35), there is no information about what happens in diabetic patients before the development of coronary atherosclerosis.

In view of recent studies that have shown that coronary endothelial dysfunction could be a predictor of CVEs (6–8), we selected diabetic patients with angiographically normal coronary arteries and without any other major risk factor, and we used CPT, a very simple method, to study the endothelial function of large coronary vessels by quantitative angiography. Our findings, which are based on a follow-up period of  $45 \pm 19$  months, show that in diabetic patients with coronary constriction, only 67.9% remained free of CVE, whereas 89.5% of diabetic patients without coronary constriction remained free of CVE (one patient developed a stable angina that required angioplasty). Moreover, major events were observed only in patients with coronary constriction (stroke, sudden cardiac death, unstable angina, and myocardial infarction). Comparable results have been reported in patients with mild coronary artery disease or with normal coronary arteriography, whose epicardial arteries and/or coronary microcirculation constricted in response to acetylcholine (7,8), a method that required intracoronary Doppler and intracoronary injection of acetylcholine.

The predictive value for CVE of constriction of the epicardial coronary arteries to CPT or to acetylcholine has been shown to be comparable in patients with coronary artery disease (6). Our results confirm that CPT is a reliable method for testing endothelial function and that it may reveal endothelial dysfunction.

In our study, endothelium-independent response to isosorbide dinitrate was normal and comparable in all of the groups (Fig. 2) and could not serve as a predictor of CVE. These results are at variance with those of Schächinger et al. (6), but similar to those in other studies (7,8). This discrepancy might be due to the se-



**Figure 3**—A: Proportion of patients with cardiac events in each group of the study population. B: proportion of patients free of cardiac events in the different groups (Kaplan-Meier analysis, log-rank P value).

verity of coronary atherosclerosis in the first study, whereas we have studied patients free of angiographically visible lesions.

Because the endothelium also plays an important role in the control of thrombogenesis, platelet activity, vascular hypertrophy and remodelling, the inflammatory process, lipid deposition, accumulation of foam cells, and the development of lipid plaques (2), our data suggest that type 2 diabetic patients with endothelial dysfunction develop atherosclerosis more rapidly, even when diabetes is treated. However, we were unable to show any difference between the different classes of antidiabetic agents, possibly because the population was too small, and probably because most of the patients were treated by more than one agent, and because during follow-up the therapy was modified to improve glycemic control, and all of the included patients had appropriate glycemic control at the end of the study.

#### Study limitations

This study is retrospective and, although the patients were carefully selected to permit conclusions on the specific cardiovascular risk of type 2 diabetes, the number of patients was relatively small, and the results remain to be confirmed by a larger prospective study. This small number of patients explains that, despite results showing that the event rate was significantly higher in group 2 than in control subjects, the threshold of significance was not reached for comparison between groups 1 and 2.

In our study, although many control subjects and diabetic patients were receiving lipid-lowering therapy at the time of coronary artery angiography, we considered these patients to have no cardiovascular risk linked to hypercholesterolemia because lipid fraction concentrations were within the normal range and comparable in diabetic patients and control subjects. Although it has been demonstrated that lipid lowering could restore a

normal coronary endothelial function, the proportion of patients with lipid-lowering therapy was comparable in the two groups of diabetic patients. On the other hand, although cholesterol components were comparable in the two groups, the LDL-to-HDL ratio was moderately higher in diabetic patients and might have played a role in their increased risk. However, we did not find differences for CVEs between patients having an LDL-to-HDL ratio  $\geq 2.00$  and those with a ratio  $< 2.00$ .

Although systolic pressure was higher in our diabetic patients, at time of the inclusion, all patients had systolic pressure  $< 140$  mmHg and diastolic pressure  $< 90$  mmHg, values that were considered normal by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure and the World Health Organization. Nevertheless, it has been shown that aggressive blood pressure control in normotensive diabetic patients may prevent the development or progression of microalbuminuria (36), which is a cardiovascular risk factor and linked to microvascular complications, suggesting endothelial dysfunction, and recommended values for diabetic patients have been lowered recently. However, we do not think that arterial pressure in our diabetic patients could explain the differences in prognosis with control subjects and between the two groups of diabetic patients because arterial pressure was not different in the two groups of diabetic patients and the proportion of patients with microalbuminuria in the two groups of diabetic patients was the same (5 in group 1 and 11 in group 2).

Selection was based on an abnormal exercise test and/or stress thallium scintigraphy and coronary arteriography, which represents only a small part of the diabetic population. Because patients with a normal exercise test and/or stress thallium scintigraphy and patients with an abnormal coronary arteriography were excluded, results cannot be extended to all diabetic patients. However, we cannot exclude angiographically undetectable atherosclerosis because intravascular ultrasound studies (37) have shown that early coronary atherosclerosis can be present despite angiographically normal vessels. However, it has been shown that there was no significant correlation between cor-

onary intravascular ultrasound results and endothelium-dependent response to acetylcholine (7). Lastly, results show that baseline and the maximal diameter of the coronary arteries were comparable in all of the groups (Table 3). Therefore, we think that concentric diffuse coronary atherosclerosis can be reasonably ruled out.

### Conclusions and clinical implications

Endothelial dysfunction of the large coronary arteries may identify a subgroup of diabetic patients at higher risk for CVEs. This information can be obtained by using CPT without pharmacological testing and intracoronary Doppler. However, because coronary arteriography cannot be used as a screening method, it would be of interest to investigate whether similar predictive information could be obtained from a peripheral noninvasive study of the endothelial function (brachial post occlusive hyperemia, for example), since it has been demonstrated that endothelial dysfunction is detectable in first-degree relatives of patients with type 2 diabetes (38) and because it has been recently shown that forearm endothelial dysfunction evidenced by intravascular acetylcholine might be a marker of future CVE in hypertensive patients (39). It would also be of interest to conduct prospective studies on agents that improve endothelial function in order to see whether they may prevent CVE.

### References

- Nathan DM: Long-term complications of diabetes mellitus. *N Engl J Med* 328: 1676–1685, 1993
- Quyyumi AA: Endothelial function in health and disease: new insights into genesis of cardiovascular disease. *Am J Med* 105 (Suppl.):S32–S39, 1998
- Vita JA, Keaney JF: Endothelial function: a barometer for cardiovascular risk? *Circulation* 106:640–642, 2002
- Nitenberg A, Valensi P, Sachs R, Dali M, Aptekar E, Attali JR: Impairment of coronary vascular reserve and Ach-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. *Diabetes* 42:1017–1025, 1993
- Nitenberg A, Paycha F, Ledoux S, Sachs R, Attali JR, Valensi P: Coronary artery responses to physiological stimuli are improved by deferoxamine but not by L-arginine in non-insulin-dependent diabetic patients with angiographically normal coronary arteries and no other risk factors. *Circulation* 97:736–743, 1998
- Schächinger V, Britten MB, Zeiher AM: Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 101:1899–1906, 2000
- Al Suwaidi J, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Lerman A: Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 101:948–954, 2000
- Halcox JP, Schenke WH, Zalos G, Minemoyer R, Prasad A, Waclawiw MA, Nour KRA, Quyyumi AA: Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 106:653–658, 2002
- Nabel EG, Ganz P, Gordon JB, Alexander RW, Selwyn AP: Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. *Circulation* 77:43–52, 1988
- Antony I, Aptekar E, Lerebours G, Nitenberg A: Coronary artery constriction caused by the cold pressor test in human hypertension. *Hypertension* 24:212–219, 1994
- Sahn DJ, DeMaria A, Kisslo J, Weyman A: Recommendations regarding quantitation in M-mode echocardiography: result of a survey of echocardiographic measurements. *Circulation* 58:1072–1083, 1978
- Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N: Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 57:450–458, 1986
- Nitenberg A, Benvenuti C, Aptekar E, Antony I, Deleuze P, Loisanche D, Cachera JP: Acetylcholine-induced constriction of angiographically normal coronary arteries is not time dependent in transplant recipients. *J Am Coll Cardiol* 22:151–158, 1993
- Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–36, 1976
- Vita JA, Treasure CB, Nabel EG, McLenachan JM, Fish RD, Yeung AC, Vekshtein VI, Selwyn AP, Ganz P: Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation* 81:491–497, 1990
- Zeiher AM, Drexler H, Wollschläger H, Just H: Modulation of coronary vasomotor tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis. *Circulation* 83:391–401, 1991
- Robertson D, Johnson GA, Robertson RM, Nies AS, Shand DG, Oates JA: Comparative assessment of stimuli that release neuronal and adrenomedullary catecholamines in man. *Circulation* 59:637–643, 1979
- Macho P, Hintze TH, Vatner SF: Regulation of large coronary arteries by increases in myocardial metabolic demands in conscious dogs. *Circ Res* 49:594–599, 1981
- Angus JA, Cocks TM, Satoh K:  $\alpha$ 2-Adrenoreceptors and endothelium-dependent relaxation in canine large arteries. *Br J Pharmacol* 88:767–777, 1986
- Drexler H, Zeiher AM, Wollschläger H, Meinertz T, Just H, Bonzel T: Flow-dependent coronary artery dilatation in humans. *Circulation* 80:466–474, 1989
- Cooke JP, Rossitch E Jr, Andon NA, Loscalzo J, Dzau VJ: Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. *J Clin Invest* 88:1663–1671, 1991
- Furchgott RF, Carvalho MH, Khan MT, Matsunaga K: Evidence for endothelium-dependent vasodilation of resistance vessels by acetylcholine. *Blood Vessels* 24:145–149, 1987
- Rubanyi GM, Romero JC, Vanhoutte PM: Flow-induced release of endothelium-derived relaxing factor. *Am J Physiol* 250 (Suppl.):H1145–H1149, 1986
- Zeiher AM, Drexler H, Wollschlaeger H, Saurbier B, Just H: Coronary vasomotion in response to sympathetic stimulation in humans: importance of the functional integrity of the endothelium. *J Am Coll Cardiol* 14:1181–1190, 1989
- Antony I, Lerebours G, Nitenberg A: Angiotensin-converting enzyme inhibition restores flow-dependent and cold pressor test-induced dilations in coronary arteries in hypertensive patients. *Circulation* 94: 3115–3122, 1996
- Nitenberg A, Ledoux S, Valensi P, Sachs R, Attali JR: Impairment of coronary microvascular dilation in response to cold-pressor induced sympathetic stimulation in type 2 diabetic patients with abnormal stress thallium imaging. *Diabetes* 50: 1180–1185, 2001
- Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D: Enhanced cellular oxidant stress by the interaction of advanced glycation end-products with their receptor/binding proteins. *J Biol Chem* 269:9889–9897, 1994
- Rubanyi GM, Vanhoutte PM: Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 250 (Suppl.):H822–H827, 1986
- Ting HH, Timini FK, Boles KS, Creager SJ, Ganz P, Creager MA: Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin dependent diabetes mellitus. *J Clin Invest* 97:22–28, 1996
- Nitenberg A, Ledoux S, Valensi P, Sachs

- R, Antony I: Coronary microvascular adaptation to myocardial metabolic demand can be restored by inhibition of iron-catalyzed formation of oxygen free radicals in type 2 diabetic patients. *Diabetes* 51: 813–818, 2002
31. Nahser PJ, Brown RE, Oskarsson H, Winniford MD, Rossen JD: Maximal coronary flow reserve and metabolic coronary vasodilation in patients with diabetes mellitus. *Circulation* 91:635–640, 1995
  32. Antony I, Lerebours G, Nitenberg A: Loss of flow-dependent coronary artery dilatation in patients with hypertension. *Circulation* 91:1624–1628, 1995
  33. Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229–234, 1998
  34. Evans JM, Wang J, Morris AD: Comparison of cardiovascular risk between patients and those who had had a myocardial infarction: cross sectional and cohort study. *BMJ* 324:939–942, 2002
  35. Vanzetto G, Halimi S, Hammoud T, Fagret D, Benhamou PY, Cordonnier D, Denis B, Machecourt J: Prediction of cardiovascular events in clinically selected high-risk NIDDM patients: prognostic value of exercise stress test and thallium-201 single-photon emission computed tomography. *Diabetes Care* 22: 19–26, 1999.
  36. Schrier RW, Estacio RO, Esler A, Mehler P: Effects of aggressive blood pressure control in normotensive type 2 diabetic patients on albuminuria, retinopathy and strokes. *Kidney Int* 61:1086–1097, 2002
  37. St Goar FG, Pinto FJ, Alderman EL, Fitzgerald PJ, Stinson EB, Billingham ME, Popp RL: Detection of coronary atherosclerosis in young adult hearts using intravascular ultrasound. *Circulation* 86: 756–763, 1992
  38. Balletshofer BM, Rittig K, Enderle MD, Volk A, Maerker E, Jacob S, Matthaei S, Rett K, Häring HU: Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 101:1780–1784, 2000
  39. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G: Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 104:191–196, 2001